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Leading Edge
Essay
Cell

Coming Full Circle—From Endless Complexity to Simplicity and Back Again

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Cell has celebrated the powers of reductionist molecular biology and its major successes for four decades. Those who have participated in cancer research during this period have witnessed wild fluctuations from times where endless inexplicable phenomenology reigned supreme to periods of reductionist triumphalism and, in recent years, to a move back to confronting the endless complexity of this disease.

In the mid-1970s, when *Cell* published its first edition, the mechanisms by which cancer started and spread were a total mystery. Half a century of cancer research had generated an enormous body of observations about the behavior of the disease, but there were essentially no insights into how the disease begins and progresses to its life-threatening conclusions.

As a result, the field of cancer research was held in ill-disguised contempt by the growing crowd of molecular biologists, geneticists, and biochemists. Even the cancer researchers had become rather disillusioned with the vast body of essentially incoherent phenomena that constituted "cancer research": as one particularly jaundiced cancer researcher told me at the time "one should never, ever confuse cancer research with science!"

The birth of *Cell* reflected the growing confidence that molecular biology would be able to answer questions that previously appeared intractable. Despite the low opinion some had of cancer research, we molecular biologists imagined that somehow we would ride in—knights on white horses—and save the day. We were, after all, reductionists, who would parse cancer cells down to their smallest molecular details and develop useful, universally applicable lessons about the mechanisms of cancer development. We would somehow develop logical order out of the phenomenological chaos that the traditional cancer researchers had been accumulating for more than half a century.

Arrogance like this is never appreciated, and so we tried to keep it under wraps. We were aware of the sensitivities of the ruling barons of cancer research and tried to be nonconfrontational. We couched our work in molecular biological terms that were unthreatening for those who had toiled for generations without making much headway into the simple questions of what cancer was and how it began. We knew, all along, that simple answers to complex questions would be greeted with mixed feelings by the large community of more traditional cancer researchers. After all, if we succeeded, we might put many of them out of business.

I suppose our self-confidence was necessary to make our way through the endless complexity represented by neoplastic disease: We needed to ignore the objections that the old-line cancer researchers repeatedly tossed into our path; they said that cancer was really much too complicated to be understood through simple molecular mechanisms. Indeed, they portrayed our reductionism as simplistic if not simple-minded.

At the same time, we were kept honest by occasional attacks from the left, from the serious geneticists who were, at the time, laying the foundation stones of modern molecular genetics. How could we pretend, they asked, to be doing serious, rigorous science if we worked with genetically ill-defined mammalian and avian cells? In their minds, only *E. coli,* yeast, and *Drosophila* genetics held the promise of yielding solid, irrefutable conclusions. This sniping from all sides cemented in my mind the reality that no matter what one does in life, it is guaranteed to be wrong in someone's eyes.

The molecular cancer story really began early in the decade—1971 to be precise—when an enormous pot of money suddenly became available for cancer research. President Nixon's War on Cancer, as it came to be called, was fueled by the conviction that cancer was ultimately a disease of infectious tumor viruses. By then, it was clear that a diverse array of viruses carrying DNA or RNA genomes were able to infect normal cells and transform them into tumor cells. Because these transformations happened in cell cultures, this suggested that the process of converting a normal cell into a tumor cell was not confined to living tissues. One could actually observe it happening in the Petri dish!

Much of the enthusiasm for launching the War on Cancer came from the independent discoveries by Howard Temin and David Baltimore that viruses known to be capable of infecting animals and triggering tumor formation carried the reverse transcriptase enzyme (Temin and Mizutani, 1970; Baltimore, 1970). In ways that are difficult to reconstruct now, some portrayed the enzyme as the key to understanding human cancer. The

argument was that relatively simple assays for this enzyme in human tumor samples would reveal the elusive retroviruses that were the underlying causes of human cancer. It seemed that an understanding of the disease of cancer was finally ripe for the picking.

It's hard to know now whether the scientific proponents of the War on Cancer (officially the National Cancer Act of 1971) really believed this notion, or only used it to persuade politicians that this enzyme provided the critical key to a definitive understanding of this disease. For whatever reason, the War was generously funded by the U.S. government, and a mad scramble ensued to find reverse transcriptase and thus the otherwise-elusive retroviruses lurking in human tumor samples.

Those working on DNA tumor viruses, such as polyoma, SV40, adenovirus, and herpesviruses, jumped on the band wagon, since the war cry had expanded: like RNA tumor viruses, viruses with DNA genomes also played a role in triggering neoplastic disease. In retrospect, few seemed deterred by the well-established observation that most types of human cancer did not represent communicable diseases.

By the mid-1970s, with rare exception, tumor virologists had come up empty-handed in their search for human retroviruses. There was some muted grumbling about overenthusiasm if not outright deception on the part of those who had inveigled the US Congress to launch the War on Cancer in the first place.

The work went on nevertheless, because of a simple inescapable fact: the ability of invading viruses to transform an infected cell dictated that relatively small viral genomes carrying small numbers of transforming genes could drive an infected cell into the neoplastic growth state. Stated differently, it appeared that small numbers of viral genes could somehow overrule the vastly larger genomes of cells, forcing the latter into a neoplastic state. This, on its own, represented a truly revolutionary concept!

Still, the notion that cancer was a disease of identifiable genes was little more than an attractive speculation. The Varmus-Bishop discovery of the *src* proto-oncogene in 1975–1976 changed that (Stehelin et al., 1976). Their work showed that normal cells carried a gene that, in principal, could be kidnapped and corrupted by a marauding retrovirus and could thereafter drive cancer formation. This represented yet another enormous leap forward, because it connected reductionist molecular biology with cell transformation. Still, its relevance to human cancer was hardly obvious, since retroviruses like Rous sarcoma virus (RSV), in which the *src* oncogene was discovered, were impossible to find in human cancers.

Looking back, it's clear that the scramble to find human retroviruses represented the major irony of the War on Cancer: it had been launched for the wrong reason, since cancer-causing human retroviruses were never found (with the exception of rare leukemias in the Caribbean and southern Japan). In the decades that followed the beginning of The War, we were able to justify this wrong-headedness because this War yielded, in ways that no one could anticipate, two enormous benefits.

By the end of the 1970s, the retrovirologists, stimulated by the Varmus-Bishop work, began to uncover a growing repertoire of proto-oncogenes residing in the vertebrate genome; these genes would soon provide a critical entrée into the mysteries of human cancer formation (Bishop, 1983). Moreover, soon after AIDS was first described in 1981, its etiologic agent—HIV—was uncovered with astonishing rapidity, a direct legacy of the retrovirology that had been developed under the aegis of the War on Cancer during the previous decade (Barré-Sinoussi et al., 1983; Gallo et al., 1983).

In my own case, I was energized by the 1973 report of Bruce Ames (Ames et al., 1973; McCann et al., 1975a, 1975b) and follow-up studies showing that the carcinogenic potency of a chemical species correlated directly with its mutagenic activity. This observation represented a compelling demonstration that cancer cells were, as some had speculated, actually mutants, and that the mutant genes carried by these cells drove malignant cell proliferation. (Only later was it clear that, Ames' work notwithstanding, most human carcinogens are actually not mutagenic, but fortunately I and others were not derailed by discrepant facts.)

That was the world into which I and other molecular biologists confidently strode 40 years ago. Very soon, it appeared we might be right: the birth of a malignant cell and its subsequent spread might indeed be the result of a few molecular events. Later on, things got more complicated again. Perhaps my colleagues and I would never have begun this work had we known how complicated things would turn out to be.

Starting in 1973, some in my group had adapted the then-recently invented Graham-van der Eb calcium phosphate transfection technique (used to introduce genes into mammalian cells; Graham and van der Eb, 1973) in order to study retrovirus replication. Indeed, as they found, transfection of viral DNA—the product of recent reverse transcription by infecting retroviruses—could yield infectious virus particles that were indistinguishable from naturally arising virus particles (Smotkin et al., 1975). We and others soon applied this procedure to studying the ability to introduce transforming genes—oncogenes—via transfection into previously untransformed cells; the readout was the appearance of foci of refractile cells in otherwise-flat monolayer cultures.

The question was whether cells that had been transformed through exposure to a mutagenic carcinogen—methylcholanthrene—contained oncogenic information in their DNA, ostensibly the direct product of mutagenesis; importantly, these chemically transformed cells gave no evidence of previous retrovirus infections. By 1979, through use of transfection, the DNA of the chemically transformed cells was indeed shown to contain transforming information (Shih et al., 1979), which was followed up 3 years later by the isolation through cloning of a transfected human bladder carcinoma gene (Shih and Weinberg, 1982; Pulciani et al., 1982; Goldfarb et al., 1982). It seemed that transfer of a single gene from cancer cells to normal cells sufficed to transform the latter.

Provocatively, this human oncogene was a homolog of the *Ras* oncogene discovered several years earlier by the tumor virologists (DeFeo et al., 1981; Parada et al., 1982; Der et al., 1982). This led to yet another simplifying notion: that a common repertoire of proto-oncogenes

residing in the mammalian genome could be activated either by roving retroviruses in animals or by chemical carcinogens in human cells.

A further simplification followed close on the heels of this realization. Thus, DNA sequencing revealed that the bladder carcinoma oncogene differed from its normal proto-oncogene counterpart by a single point mutation (Tabin et al., 1982; Reddy et al., 1982; Taparowsky et al., 1982). For a brief moment in 1982, there was the illusion that cancer was as simple as it possibly could be—a normal cell differed from its neoplastic counterpart by one base out of three billion!

From the point of view of the reductionist hoping that a small number of molecular events might explain cancer, things went downhill from there for the next 30 years. Within weeks of the announcement of the *RAS* point mutation, there were those who said that things could not be that simple. John Cairns, who had studied the detailed biology of human cancer development (Cairns, 1978), pointed out, correctly, that cancer development was a multistep process, likely involving a succession of rate-limiting steps, specifically stochastically occurring rare oncogenic mutations. A single somatic mutation was unlikely to explain this complexity.

Nonetheless, perhaps the number of these essential mutation-driven steps was relatively small. Indeed, by 1983, two mutant genes, collaborating with one another, were found to be capable of transforming fully normal cells into tumor cells (Land et al., 1983; Ruley, 1983). But even this was an illusion, as only became apparent years later: While experimental transformation of rodent cells indicated two oncogenes sufficed for transformation, the corresponding human cells, which had long proven quite refractory to experimental transformation, required as many as five distinct introduced mutant genes, perhaps even more (Hahn et al., 1999). So two simple notions—that small numbers of genes would suffice to transform cancer cells, and that all mammalian cells would follow the same set of genetic rules during the course of neoplastic transformation—were undermined. Still, these were observations that could somehow be accommodated in the thinking of reductionists intent on puzzling out the genetic logic of cancer pathogenesis.

Soon a new skirmish broke out. This one focused on which classes of mutant genes were really important for cell transformation: oncogenes or the tumor suppressor genes? A vocal advocate of tumor suppressor genes—indeed a founder of this field—dismissed the oncogene gold rush of the mid-1980s as an act of lunacy, a band-wagon effect…likening us to lemmings rushing en masse over the edges of cliffs. This was another useful lesson, at least for me. I came to appreciate that the strongly held opinions of widely respected senior professors should be taken with large grains of salt.

As it turned out, the importance of both classes of genes soon became apparent. This notion acquired traction from the Vogelstein work of 1989 demonstrating a specific set of genetic changes associated with distinct histopathological stages of colorectal cancer pathogenesis (Vogelstein et al., 1989; Fearon and Vogelstein, 1990). The greater the degree of progression a tumor exhibited, the larger were the number of somatic mutations affecting both oncogenes and tumor suppressor genes. Hence, both types of mutant genes seemed to be important to tumor development and both types coexisted within individual cancer cells.

Most who were aware of this genetic flow chart of tumor development read far more into its conclusions than its authors had intended. Some seized on the work as evidence that a specific sequence of mutations in a defined set of genes was responsible for the appearance of most human colorectal carcinomas. In truth, the research revealed nothing more than a probabilistic trend, with *APC* mutations being, almost invariably, the first step, followed by mutations of other genes, such as *K-RAS* and *p53*, affecting some colon tumors but not others. Moreover, with the passage of time, the order of acquisition of these secondary mutations was found to vary from one colon tumor to the next, and many were even found to lack any sign of mutant *RAS* oncogenes. Indeed, it became clear that colorectal carcinomas follow highly variable genetic paths *en route* from normalcy to full-fledged neoplasia, so a simple linear narrative describing the development of this class of tumors no longer seemed to hold water.

All the while, the 1980s and 1990s witnessed an explosive increase in the roster of oncogene and tumor suppressor genes, many of which were implicated in human cancer development. These provided additional indication that cancer development would not be simple. It became clear that the identities of mutant cancer-causing genes varied dramatically from one type of tumor to the next. Moreover, even within a given type of cancer, such as the much-studied colorectal carcinomas cited above, there were no uniform successions of genetic change. Instead, each tumor seemed to represent a unique experiment of nature, acquiring a unique set of mutant genes and in an unpredictable chronological order.

Keenly aware of these unsettling trends, and holding out hope against hope, Doug Hanahan and I reasoned in 1999 that there must nevertheless be some underlying order beneath the increasingly complex, if not chaotic, genetic phenomenology of cancer. Cancers seemed to present myriad different faces to the world, but there must be, we reasoned, certain underlying commonalities. After all, cells only harbor a finite array of phenotypes and intracellular signaling circuits. In principle, one way to depict these commonalities would be to enumerate the specific intracellular signaling channels that are deregulated in most if not all types of human cancers. However, at the time, as is still the case 15 years later, our understanding of how most of these signal-processing circuits actually operate was fragmentary.

For this reason, we side-stepped the issues of signal transduction biochemistry and focused instead on biology—on the phenotypes of cancer cells and the tumors that they formed (Hanahan and Weinberg, 2000). There lay underlying order, we reasoned, hiding beneath the complex behaviors of cancer cells. We ultimately converged on six biological traits—"hallmarks" as Doug called them—that might well encompass almost all of the biology of all types of human tumors.

We fully expected the review article that we cobbled together to disappear, sinking quickly like a stone thrown into a

quiet pond. Like most of what we wrote and write, we felt that, while this review might serve to clarify our own thinking, it was unlikely to resonate with the diverse community of cancer researchers, many of whom would dismiss it as simplistic. As it turned out, we were wrong. Thousands of articles referred to this review in the decade after it appeared—a tribute not to its writing, but instead to the profound need of so many colleagues to find some unifying themes among the ever-growing mass of observations.

We renewed this attempt to find underlying order a decade later in 2011, when we once again proposed the role of hallmarks in helping conceptualize how most human cancers arise (Hanahan and Weinberg, 2011). By then, the six hallmarks had grown to eight. Still, the overall scheme seemed sound. Maybe human cancers really did manifest a relatively small set of features that governed their behavior, even if each of those features could be caused by still-unknown molecular events.

There also seemed hope for order in one of the more rapidly moving areas of cancer research, which addresses the biological processes of invasion and metastasis. Carcinoma cells seemed to employ a relatively small number of shared mechanisms in order to escape primary tumors and travel to distant sites. Order seemed to be emerging out of this lethal chaos (Thiery, 2002). Then reality again reared its head: once disseminated cancer cells enter into unfamiliar, often inhospitable tissue microenvironments, no simple rules seemed to describe their mechanisms of adaptation—each type of cancer cell and each distinct destination site appeared to require its own set of adaptations, which are acquired only with great difficulty, fortunately for the cancer patient.

Starting in 2000, the few voices proposing order and simplicity in cancer research became increasingly drowned out by a far more powerful chorus. The generation of enormous data sets had by then become routine and highly prized, at least by some, as indicators of scientific productivity. When I was a graduate student, the evaluation of a single gel electrophoresis channel required half a day's work; by 2000, thousands of such channels could be run and analyzed in this time. Expression array analyses could now be performed to analyze the expression of thousands of genes in thousands of tumors. Genome sequencing also came of age and documented myriad mutations afflicting individual cancer cell genomes.

For many, generating large data sets became an almost-addictive undertaking. If two interacting entities—proteins for example—were critical to a well-established biological process, imagine what studying two thousand proteins via interactome analyses would yield! And so, we entered, almost unconsciously, into the epoch of "omics"—studying genomes, transcriptomes, proteomes, epigenomes, kinomes, methylomes, glycomes, and matrisomes—each one of which encompasses staggering amounts of accumulated information. The relative ease of generating vast amounts of data became almost irresistible.

The currently embraced notion is that a complex system can only be understood if all of its moving parts are analyzed in one sweeping overview. Such holistic analyses should ideally describe the complex reality of actual biological systems, including that of cancer cells. More observational data should bring more insights, as it did decades earlier. All one needs—so the doctrine goes—are some computational algorithms to distill these data sets into simple, accessible take-home lessons that would provide mechanistic insights into how complex systems actually operate, including how cancer cells arise and how they respond to therapeutic attack.

Amusingly, the same overconfident presumption that my friends and I showed toward the old-line cancer researchers in the 1970s is palpable once again today, now being exhibited by former physicists and mathematicians and bioinformaticians: just tell us about the working parts, they say, and we will explain, indeed predict, how the complex machine—a human cell—works!

In truth, the construction of truly useful algorithms still seems to lie in the distant future. While data mining, as it's now called, occasionally flags one or another highly interesting gene or protein, the use of entire data sets to rationalize how and why a cancer cell behaves as it does is still far beyond our reach. At most, one can develop correlations between certain complex data sets (e.g., expression array analyses) and prognosis, i.e., future behavior in the oncology clinic. The gaping distance between these data sets and a true understanding of cancer biology is illustrated by the amusing fact that two distinct expression array analyses of cells in breast cancers have been found to be equally useful in predicting future clinical behavior of these tumors but contain almost no genes and thus proteins in common (Ein-Dor et al., 2005; Fan et al., 2006).

Beyond these complex, currently intractable large data sets, there are problems that cancer researchers haven't even begun to confront. How do the transcriptomes of cancer cells interact with their mutant genomes to orchestrate cancer cell behavior? How do the differentiation programs of the normal cells-of-origin influence the behavior of their neoplastic descendants that have sustained large numbers of genetic and epigenetic changes? How do the multiple distinct cell types that form the tumor microenvironment (composed of inflammatory and immune cells and cells forming the microvessels) intercommunicate with one another and influence the behavior of nearby neoplastic cells? Physicists have wrestled unsuccessfully with the three-body problem. What will become of us who try to deal with eight or ten distinct independent agents, each a distinct stromal cell type that is recruited into a tumor and interacts bidirectionally with the other recent recruits?

Then there is the nettlesome problem of multistep tumor progression: cancer is a moving target, and whatever interactions operate at one stage of tumor progression are likely to change during the next one, so that multiple solutions will need to be worked out for individual tumors. Even within an individual tumor at a single stage of progression, there is an entirely new dimension of complexity: deep-sequencing analyses of tumor DNAs now indicate multiple, genetically distinct subpopulations whose representation seems to vary dramatically from one stage of tumor progression to another.

The data that we now generate overwhelm our abilities of interpretation, and the attempts of the new discipline of "systems biology" to address this

shortfall have to date produced few insights into cancer biology beyond those revealed by simple, home-grown intuition. The coupling between observational data and biological insight is frayed if not broken.

We lack the conceptual paradigms and computational strategies for dealing with this complexity. And equally painful, we don't know how to integrate individual data sets, such as those deriving from cancer genome analyses, with other, equally important data sets, such as proteomics. This is most frustrating, since it is becoming increasingly apparent that a precise and truly useful understanding of the behavior of individual cancer cells and the tumors that they form will only come once we are able to integrate and then distill these data.

So, perhaps ironically, we have come full circle, beginning in a period when vast amounts of cancer research data yielded little insight into underlying mechanisms to a period (1980–2000) when a flurry of molecular and genetic research gave hope that cancer really could be understood through simple and logical reductionist thinking, and finally to our current dilemma. Once again, we can't really assimilate and interpret most of the data that we accumulate.

How will all this play out? I wouldn't pretend to know. It's a job, as one says on these occasions, for the next generation. Passing the buck like this is an enormously liberating experience, and so I'll keep on doing it!

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